MANUFACTURING AND TESTING OF POLYCLONAL ANTIBODY PREPARATIONS

Jenny Mellquist-Riemenschneider, Ph.D.
Division of Hematology, CBER, FDA

Introduction and background-1

Polyclonal antibody treatments have been used in the prevention of diseases for decades…

Diphtheria antitoxin

Emil von Behring

Introduction and background-2

Indications

- **Prevention of a variety of bacterial and viral diseases**
  - **Immune deficient people**
  - **Immune competent people exposed to certain pathogens (HAV, HBV, tetanus, etc.)**
- **Prevention newborn hemolytic disease (Rho(D) IG)**
- **Immune modulation (ITP)**
- **Antitoxins(botulism, diphtheria, snake and spider venoms)**

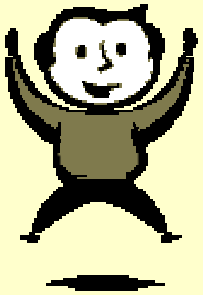
Introduction and background-3

Manufacturing

- **The process of obtaining antibodies from plasma is a complex, multi-step process (Cold ethanol precipitation, etc.).**
- **Economy of scale and need for wide spectrum of antibody specificities requires a large (≥ 1000 donor) starting plasma pool (may be less for hyperimmunes).**

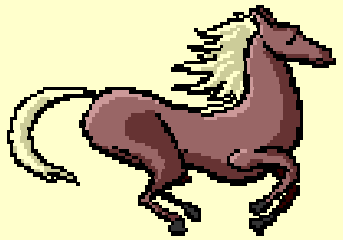
Safety	**Source**
Viral transmission	**Human**
Adverse reactions	**Animal-derived**

Considerations with human or animal-derived IG products



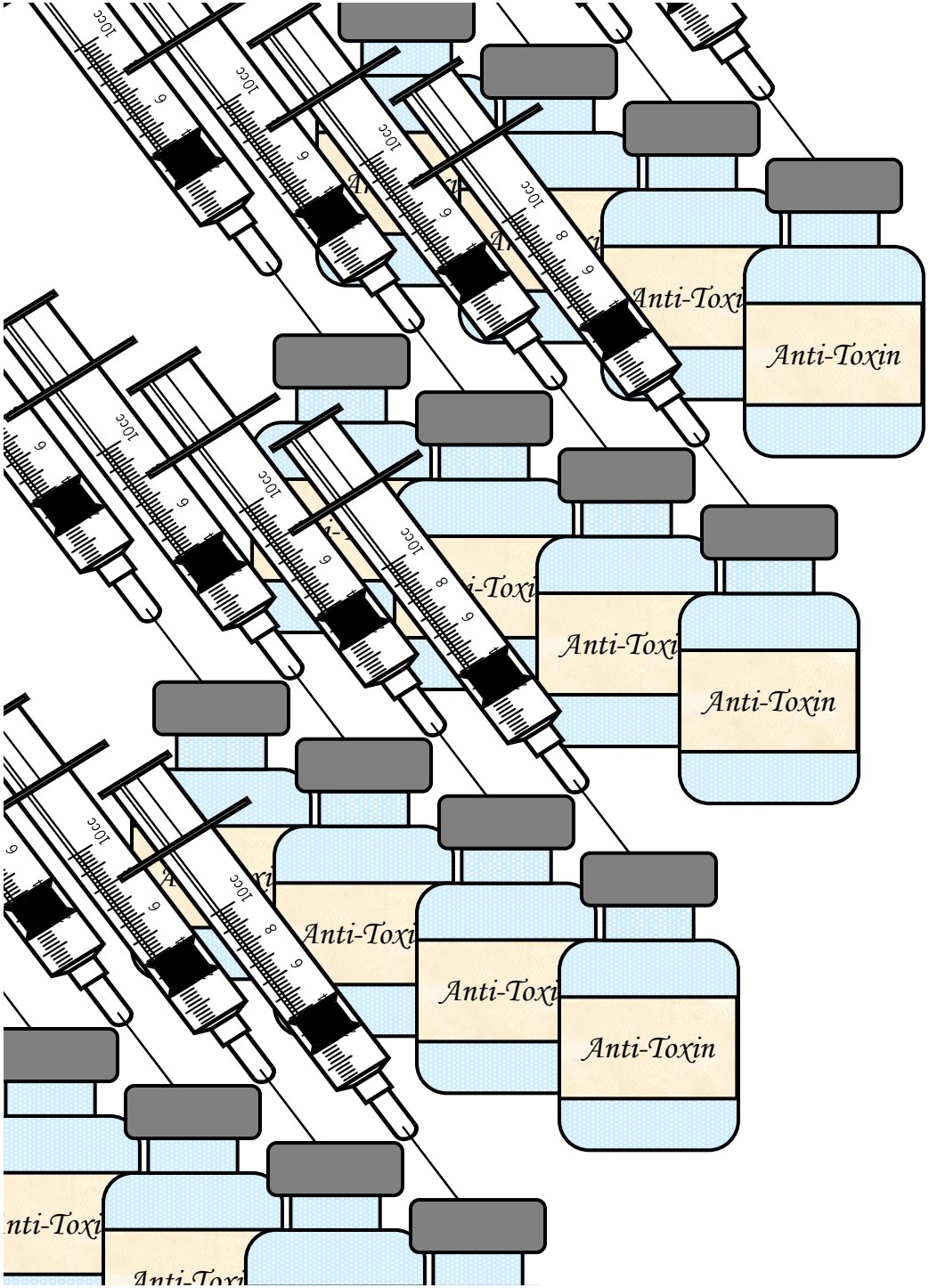
Human

- **Transmissible human diseases (e.g. HCV)**
- **Unwanted antibodies (e.g. anti-D, isoagglutinins)**
- **Availability of donors (for hyperimmunes)**



Animal

- **Immunogenicity of immune globulins**
- **Enzymatic digestion of the Fc region of the IgG molecule (despeciation)**
- **Immunogenicity of other animal proteins in the product (present in trace amounts)**
- **Need for testing for hypersensitivity/desensitization**
- **Zoonotic infectious agents (e.g. WNV, rabies)**



Historical experience with polyclonal immune globulins

Historical experience with human and animal immune globulins for potential counterterrorism use

- Vaccinia Immune Globulin (VIG)
  Human derived
- Botulism antitoxin (BAT-equine)
  Horse derived
- Licensed prior to 1980
- Modern efficacy studies were not performed
- Requirements for licensure have changed..

Human immune globulins

Typical indications

Prophylaxis

Immune globulins are used typically in prophylaxis situations: (e.g. IGIV, HBIG, TIG, CMVIGIV, RSVIGIV)

Treatment

Also used as treatment (e.g. BIG, TIG, COUNTERRORISM)

General IG products

- **IG is given by IM injection**
- **IGIV is given by IV injection**

Specific IG/IGIV products (hyperimmune)

-Rabies IG
-Hepatitis B IG
-Varicella-Zoster IG
-Tetanus IG
-Rho(D) IG(IV)
-Botulism IG(IV)
-Respiratory Syncytial Virus IGIV
-Cytomegalovirus IGIV

Source material for human immune globulins-1

Normal blood or plasma donors

Recovered plasma- is made by separating plasma in a donation of whole blood from other components

Source plasma-the process of removing whole blood and separating red blood cells from plasma. The red cells are returned to the donor and the plasma is retained for use

Select individuals with preexisting immunity

- **Routine prior immunization: tetanus**
- **Antibody titers from previous infections: CMV, RSV, varicella**
- **Rh(-) women exposed to Rh(+) pregnancies**

Source material for human immune globulins-2

Vaccinees in active immunization programs-examples:

- **Rabies-vaccinated (RIG)**
- **Rho(D)+ RBC-vaccinated men (anti-D),**
- **HBV-vaccinated (HB IG)**
- **Special immunization programs for laboratory workers (VIGIV)**

Military donors

Preventative immunization prior to active duty in endemic high risk areas.

Human donor screening -1

General issues

Uniform donor screening questionnaire (**examples**)

Today:	Feeling well, taking medications?
Past 48h:	Taken aspirin?
Past week:	Fever?
Past 8 wks:	Donated blood? Had vaccinations?
Past 12 months:	Blood transfusion/transplant? Sexual contact with HIV? Tattoo or piercing? Treated for syphilis or gonorrhea?
1980-1996:	Spent time that adds up to 3 months or more in UK?
1980-present:	Spent time in Europe that adds up to 5 yrs or more? Receive blood transfusion in the UK?
Ever:	Injection needle drugs, + for HIV, received dura mater graft, had cancer, have relatives w/ CJD?

http://www.fda.gov/cber/gdlns/donorhistques.htm

Human donor screening -2

Specific concerns for collection of plasma for Counterterrorism (CT) products

- Live vaccines - is there potential viremia with the vaccine?
- Are other IND vaccines co-administered?
- Science-based decisions are made by FDA through individual assessments of each situation.

Human donor testing

Donor test requirements for human blood and blood components-CFR 610.40 (a) (1-6)

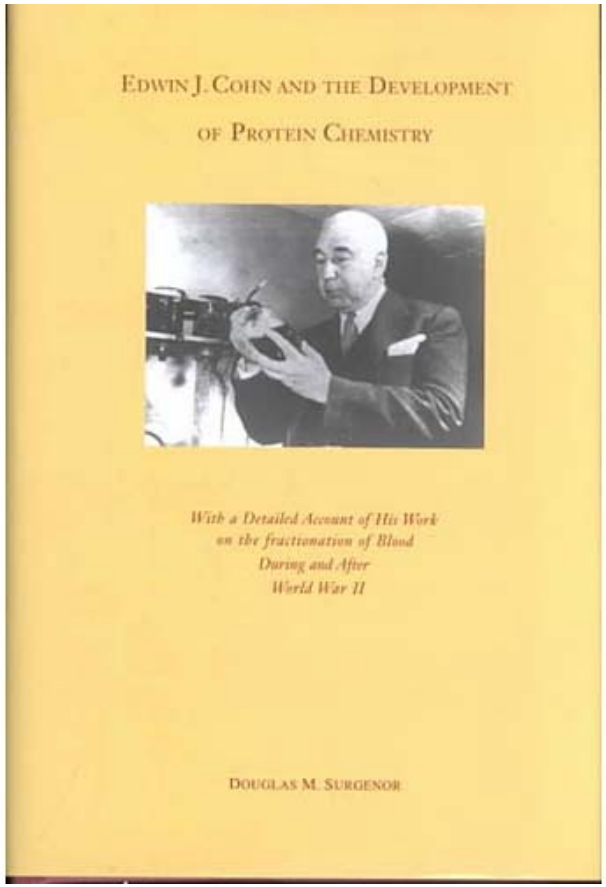
"You…must test each donation of human blood or blood component intended for use in preparing a product…for evidence of infection due to the following communicable disease agents: HIV-1, HIV-2, HBV, HCV, HTLV-1, and HTLV-2…

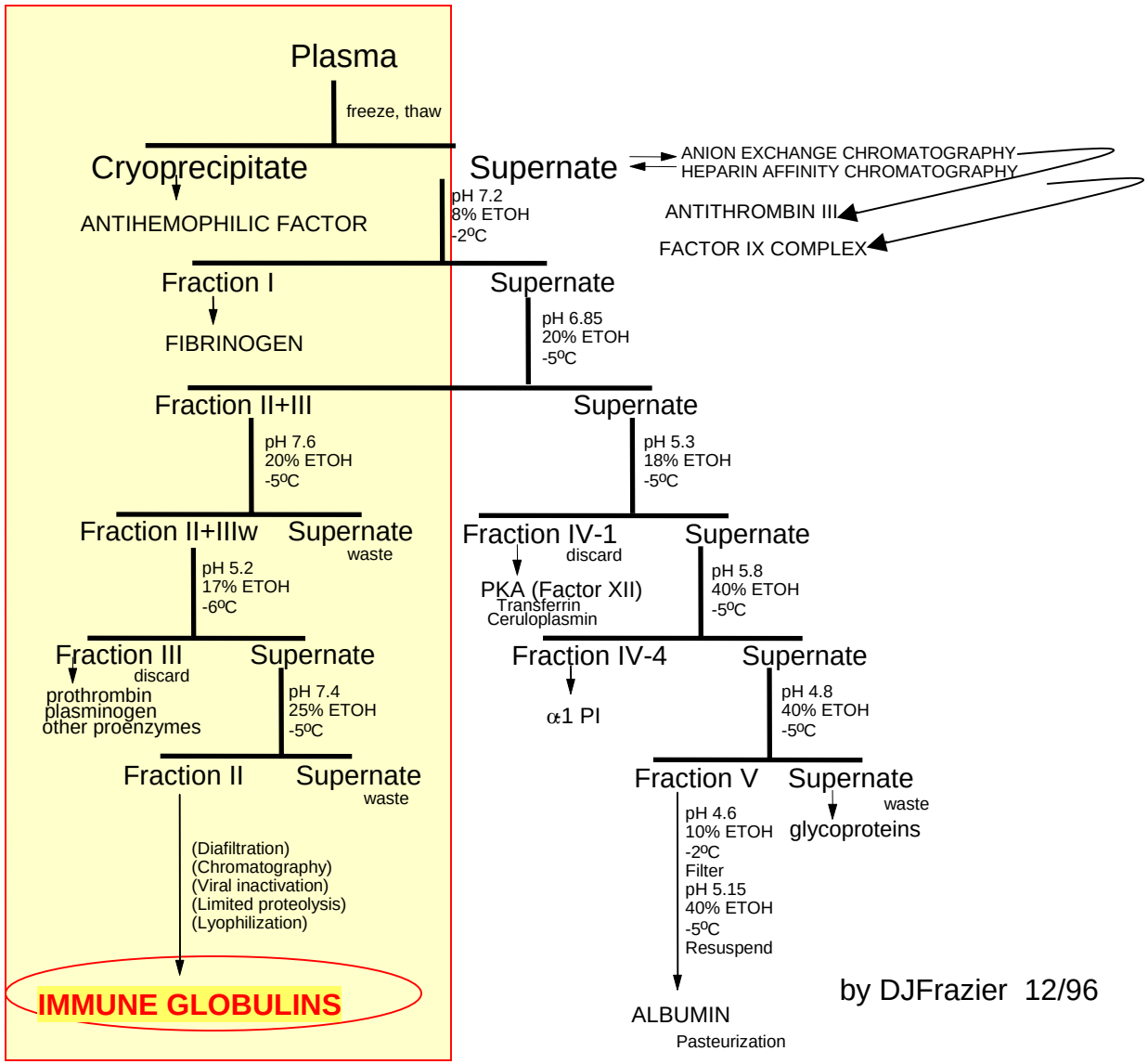
…and…[syphilis CFR 640(I)]."

Other:
e.g. West Nile Virus (under IND)

Human immune globulin manufacturing

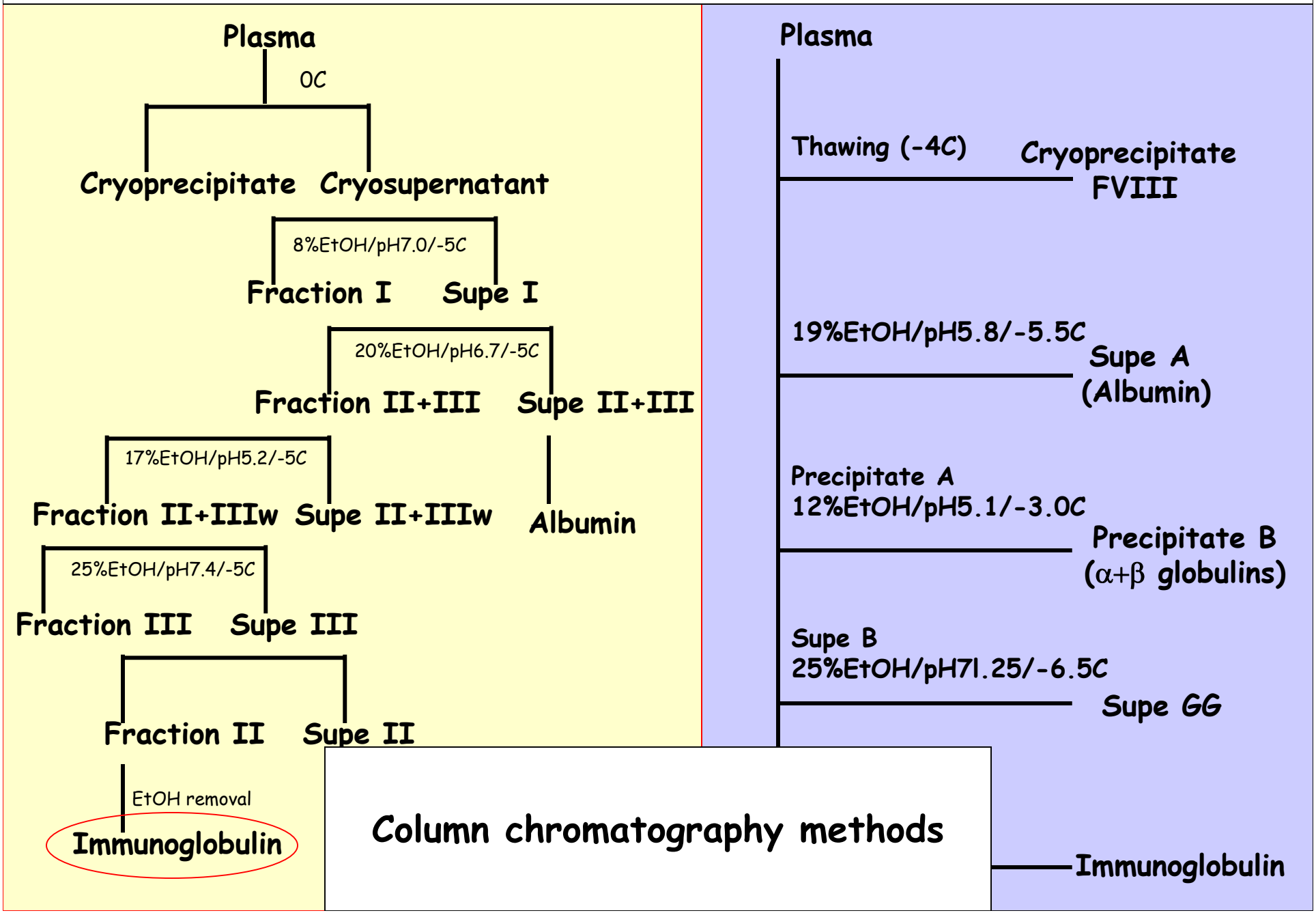
Cohn-Oncley method



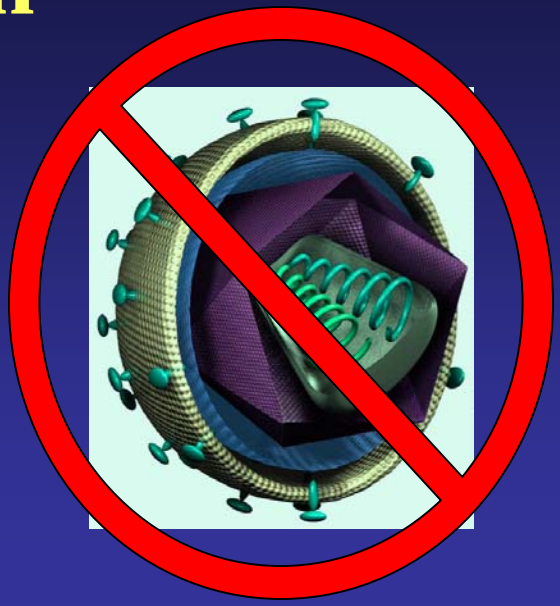


by DJFrazier 12/96

Comparison of Cohn and Kistler-Nischmann fractionation processes



Viral inactivation



Methods in fractionation process

- Partitioning during
  - fractionation
  - precipitations
  - column chromatography

Intentional viral clearance steps

- Solvent/Detergent
- Caprylate
- Heat treatment
- Nanofiltration
- Low pH

Multiple steps:

Typically recommend two orthogonal steps to clear each type of virus (enveloped, non-enveloped, sensitive, resistant, etc.).

Validation studies:

Must show clearance of the actual virus when possible.
Model (similar) viruses can be used when this is not possible.

Adverse reactions

Common and uncommon AEs:

Pyrexia
Dyspnea
Abdominal pain
Rigors, Tremor
Hypotension
Backpain
Bronchospasm

Cyanosis
Epidermolysis
Pancytopenia
Thrombosis
Pulmonary edema
Leukopenia
Hepatic dysfunction
Seizures
Hypoxemia
Erythema multiforme
Aseptic meningitis
Acute renal failure
Transfusion related acute lung injury (TRALI)

Specific:

Anaphylactoid reactions

- **Presence of aggregated Ig (measured by ACA)**

Hypotensive reactions

- **Presence of PKA, kallikrein**

CFR-required lot release testing for human immune globulins

29 CFR 610.10	Potency
29 CFR 610.11	General safety
29 CFR 610.12	Sterility (bulk, final container, retests)
29 CFR 610.13	Purity (residual moisture, pyrogen)
29 CFR 610.14	Identity
29 CFR 610.15	Constituent materials (preservatives, diluents)
29 CFR 610.11	General safety
29 CFR 610.16	Total solids in serums (incl. antitoxin)
29 CFR 610.21	Limits of potency (DAT, TAT, TIG)
29 CFR 610.100	Subpart J-Immune Globulin (human)

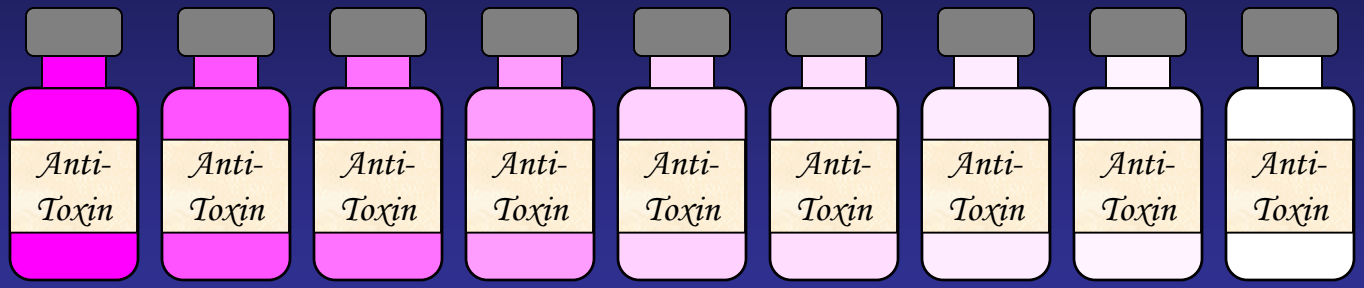
source material, additives
heat stability, microbial contamination
pH, turbidity, date of manufacture
labeling, processing mechanism, sterilization
final solution, protein composition, potency

Additional release testing typically requested for human immune globulins

Molecular distribution
- •Fragments
- •Monomers
- •Dimers
- •Aggregates

Potency (specific if hyperimmune)
Stabilizer concentration
Residuals (e.g. solvent/detergent)
Identity tests
- •Immuno-electrophoresis
- •Isoagglutinins
- •Protein concentration

Examples of stability testing parameters



- Appearance
- Total protein
- Excipient (e.g. sucrose)
- Purity (IgG)
- Fragments
- Monomer and Dimers
- Aggregates
- Residual moisture
- Solubility
- ACA
- PKA
- Sterility
- Anti HBs, HAV, Polio, Measles, DAT, Parvo
- Fc Function

Typical testing schedule at 0, 3, 6, 9, 12, 24, 36 months, etc.

Other potential sources of polyclonal immune globulin products…

Considerations of source material for animal-derived immune globulins

Immunogenicity

- Immune globulins
- Other proteins in the product (impurities)
  - Need for testing for hypersensitivity
  - Desensitization

Despeciation

- Digestion of the Fc region of the IgG molecule (to Fab or Fab'2 fragments)

Zoonotic infectious agents (examples)

- VEE, WEE, EEE
- Rabies
- WNV

Considerations of animal husbandry for animal-derived immune globulins-1

CFR 600.11 Physical establishment, equipment, animals, and care

Part (c): Laboratory and bleeding rooms

Rooms used for the processing of products, including bleeding rooms, shall be kept free of flies and vermin. Such rooms shall be so constructed as to insure freedom from dust, smoke, and other deleterious substances and to permit thorough cleaning and disinfection. Rooms for animal injection and bleeding, and rooms for smallpox vaccine animals, shall be disinfected and be provided with necessary water, electrical and other services.

Part (d): Animal quarters and stables

Animal quarters, stables, and food storage areas shall be of appropriate construction, fly-proofed, adequately lighted and ventilated, and maintained in a clean, vermin-free and sanitary condition. No manure or refuse shall be on the premises, nor shall the establishment be located in close proximity to off-property manure or refuse storage capable engendering fly breeding.

Considerations of animal husbandry for animal-derived immune globulins-2

Part (f): Animals used in manufacture

(1) Care of animals used in manufacturing:
Caretakers and attendants for animals used in the manufacture of products shall be sufficient in number and have adequate experience to insure adequate care. Animal quarters and cages shall be kept in sanitary condition. Animals on on production shall be inspected daily to observe response to production procedures. Animals that become ill for reasons not related to production shall be isolated…Competent veterinary care shall be provided as needed.

(2) Quarantine of animals:
No animal shall be used in processing unless kept under competent daily inspection and preliminary quarantine for a period of at least 7 days before use, or as otherwise provided…

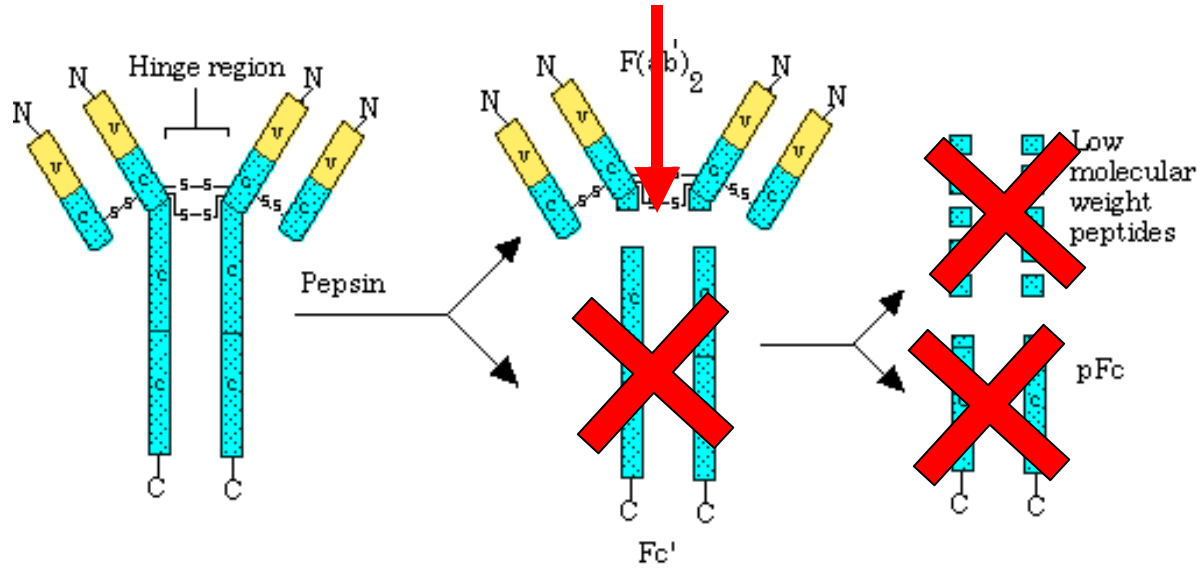
(3) Immunization against tetanus:
Horses and other animals susceptible to tetanus, that are used in the processing steps of the manufacture of biological products, shall be treated adequately to maintain immunity to tetanus.

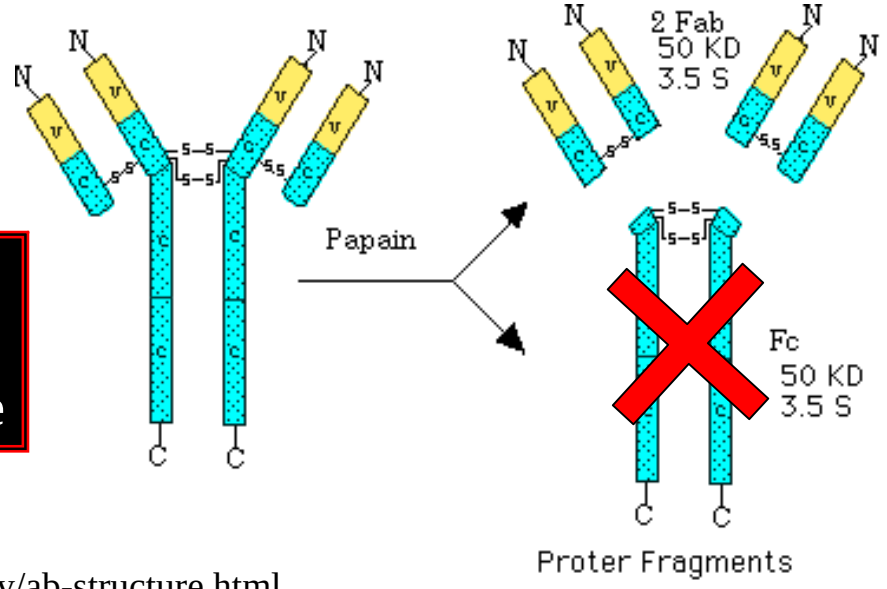
Considerations of animal husbandry for animal-derived immune globulins-3

(4) Immunization and bleeding of animals used as a source of products:
Toxins or other nonviable antigens administered in the immunization of animals used in the manufacture of products shall be sterile. Viable antigens, when used, shall be free of contaminants, as determined by appropriate tests prior to use. Injections shall not be made into horses within six inches of bleedings site. Horses shall not be bled for manufacturing purposes while showing persistent general reaction or local reaction near the site of bleeding. Blood shall not be used if it was drawn within 5 days of injecting the animals with viable microorganisms. Animals shall not be bled for manufacturing purposes when they have an intercurrent disease. Blood intended for use as a source of biological product shall be collected in a clean, sterile vessels. When the product is intended for sue by infection, such vessels shall also be pyrogen free.

(5) Reporting of certain diseases:
In cases of actual or suspected infection with FMD, glanders, tetanus, anthrax, gas gangrene, equine infectious anemia, equine encephalomyelitis, or any of the pock diseases…intended for use in the manufacture of product, the manufacturer shall immediately notify CBER.

Despeciation of animal-derived immune globulins

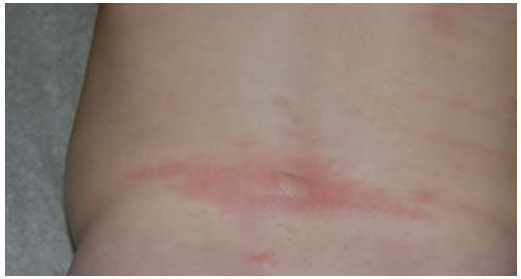




Time
Temperature
Amount of enzyme

http://ntri.tamuk.edu/immunology/ab-structure.html

Need for testing for hypersensitivity/desensitization with animal-derived immune globulins



Type I Hypersensitivity

- Wheal & erythema (wheal & flare) reaction
- Labored breathing, laryngeal edema, decreased blood pressure, hives, rapid heart-rate, anaphylactic shock (systemic)

Rapid Desensitization

- Inject very small quantity of antigen & gradually increase dose
- Mechanism unknown

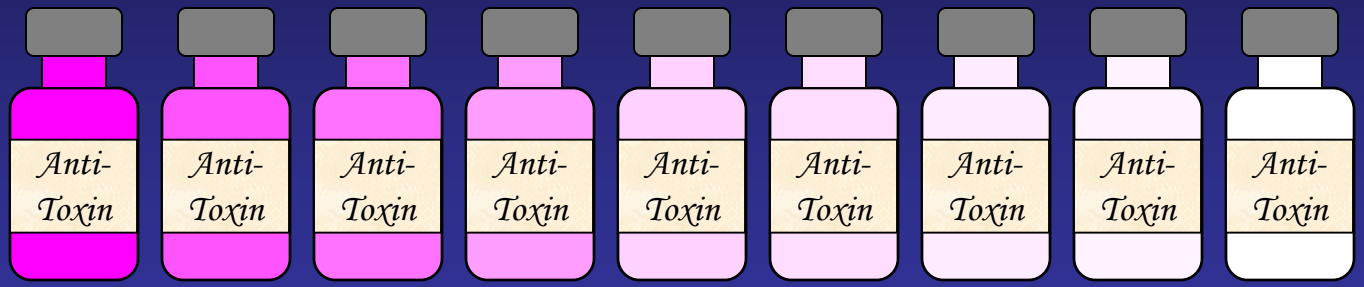
Requirement?

We have required this type of testing for animal-derived products in the past

Example lot release testing for animal-derived immune globulins

- Potency
- Purity (CE)
- Integrity by HPLC
  - Fragments
  - Monomers
  - Dimers
  - Aggregates
- Conductivity
- Total protein
- Stabilizer concentration
- Moisture
- Sterility
- Endotoxin
- Identity
- Appearance
- pH

Examples of stability testing parameters



- pH
- Physical Appearance
- Preservative (e.g. phenol)
- Potency (or potencies)
- Integrity (HPLC)
    - monomers, dimers, aggregates, fragments
- Sterility

Potential hurdles in the licensure of polyclonal immune globulins for counterterrorism

Efficacy studies

- Not possible in absence of clinical illness ("pre-event")
- Alternative strategies and phase IV commitments

Clinical safety studies

- Often possible in normal volunteers
- Adverse events/PK profile

Current strategies for the licensure of polyclonal immune globulins for counterterrorism

- **Animal Rule**
  (21 CFR 601.90-91)

- **Accelerated Approval designation**

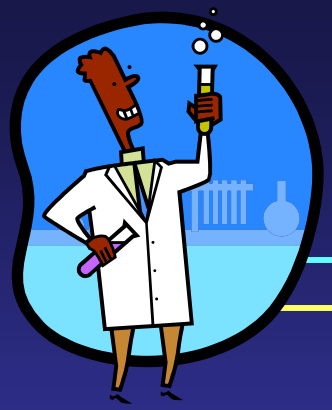
  (21 CFR 601.40 – 601.46)

  –expedited availability of product

  – Phase IV commitments to human surrogate marker validation/efficacy

Summary

- Polyclonal antibodies have the advantage of having multiple specificities against a particular antigen.
- Large amounts of plasma for the manufacture of immune globulins can be easy to obtain.
- There are multiple sources for polyclonal antibodies (human, animal).
- Plasma fractionation is a well-studied process and has been employed for decades.
- Transmissible diseases are of utmost concern and manufacturing processes must ensure that viral inactivation steps are effective.



Thanks to…

Mr. Douglas Frazier
Biologist, LPD, DH, OBRR, CBER, FDA

Dr. Dorothy Scott
Chief, LPD, DH, OBRR, CBER, FDA

Dr. Basil (Dov) Golding
Director, Division Hematology, OBRR, CBER, FDA